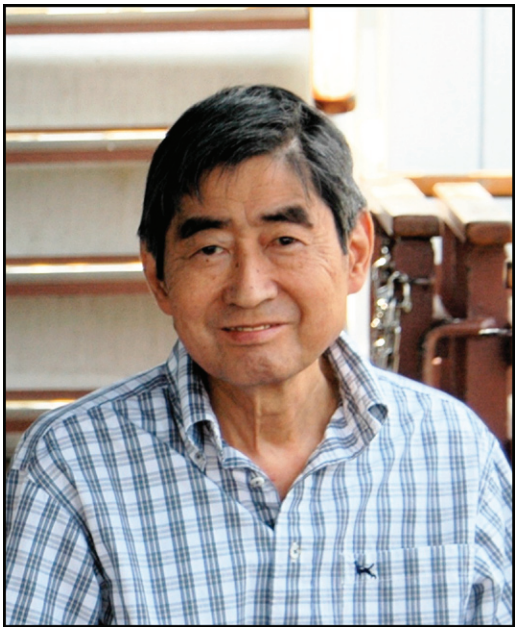
Hidesaburo Hanafusa 1929–2009

On Sunday, March 15, 2009, Hidesaburo Hanafusa died in Osaka, Japan. He was 79. With his passing, the scientific community lost a giant whose monumental achievements were matched only by his dignity, humility, honesty, and generosity to collaborators and competitors alike. Scores of former trainees lost a beloved mentor, colleague, and friend, and his family lost a devoted husband, father, and grandfather. During a career that spanned six decades, two centuries, three continents, and several cultures, Hanafusa was a pioneer in virology and molecular oncology. "Saburo" means third son, and the prefix "Hide" embellishes this with excellence and superiority. It was completely within character that he preferred to be known to students, friends, and colleagues simply as "Saburo." However, excellence and superiority shine through his nearly 300 publications notable for their originality and experimental precision; he made foundational contributions to our understanding of retroviral genetics, signal transduction, and oncogenesis. His work helped lay the groundwork for the so-called Oncogene Revolution that has begun to dramatically alter approaches to cancer therapy. His laboratory also produced several generations of investigators who have made important contributions to many fields. He will be sorely missed.

Saburo was born and educated in Japan. He had a hardscrabble upbringing because of the conditions in Japan during and after World War II. Despite these hardships, intellectual pursuits were central to the family, and Saburo became the avatar of these aspirations. He received his PhD in biochemistry in 1960 from Osaka University, where he also met his late first wife, Teruko, the only woman in his class. In addition to their enduring marriage, the couple forged a powerful scientific partnership, initially working on poxvirus replication.

By 1961, their interests had shifted to oncogenic retroviruses, and they left Osaka for Harry Rubin's lab at UC Berkeley to work on Rous sarcoma virus (RSV), an RNA tumor virus that induces sarcomas in chickens. The startling discovery of a virus that causes cancer was made by Peyton Rous in 1911, but the mechanism of RSV-mediated transformation remained an unsolved problem. Because we are living in a time when hundreds of human cancer genes have been defined, proteins can be sequenced in an afternoon, and genomes sequenced in a week, it is perhaps difficult to appreciate how little was known about the molecular basis of cancer and how crude were the tools available to the Hanafusas when they arrived to work in Rubin's lab.

Shortly before their arrival, Rubin and the late Howard Temin had made a major breakthrough with their focus-formation assay, in which "foci" of round transformed cells would appear in flat monolayer cultures of chick embryo fibroblasts infected at limiting dilution. This assay allowed viral replication and transformation to be quantified. Peter Vogt and Rubin then found that when stocks of the Bryan high-titer strain of RSV were diluted, another virus could be isolated (termed Rous-associated virus; RAV). RAV did not form foci or sarcomas but could interfere with foci and tumor formation induced by RSV. In experiments elegant in their simplicity, but supremely important for the future development of the field, Saburo and Teruko infected cells with highly dilute Bryan-RSV and overlaid the infected cultures with agar containing anti-RAV antibodies (Hanafusa et al., 1963). They then picked single foci of transformed cells and found that some foci did not produce infectious virus. Infectious transforming virus was subsequently recovered by super-infecting these transformed cells with RAV. The inescapable conclusion was that the Bryan-RSV was actually a mixture of one replication-defective virus capable of causing transformation and a helper virus (RAV) that provided a function essential for viral replication but not for transformation. The Hanafusas subsequently found this essential factor to be the RAV envelope glycoprotein (Hanafusa et al., 1964). These landmark studies established that transformation was genetically distinct from viral replication, laid the groundwork for future studies of the genetic content of retroviruses, and most importantly, strongly suggested the existence of viral oncogenes. The Hanafusas' finding that defective retroviruses can transfer specific genetic information is the conceptual underpinning for the now ubiquitous use of retroviral and lentiviral vectors.



Hidesaburo Hanafusa
Photo courtesy of Kei Hanafusa.

After a brief sabbatical in France, Saburo returned to the US in 1966 in his first independent position, at the Public Health Research Institute of New York. Several major contributions followed. First, in a remarkable series of experiments aimed at deciphering the details of RSV replication in fibroblasts derived from

different chicken embryos, Teruko and Saburo discovered "chick helper factor," providing early evidence for endogenous retroviruses (Hanafusa et al., 1970a, 1970b).

Soon, however, the attention of the Hanafusas and other major retrovirology labs turned to the fundamental question of elucidating the mechanism of cell transformation by RSV. The first step was to develop key reagents: two types of transformation-defective RSV mutants isolated by Saburo and the labs of Steve Martin, Vogt, and Peter Duesberg. Temperature-sensitive RSV mutants could replicate without transforming cells at the nonpermissive temperature but triggered transformation rapidly upon a shift to the permissive temperature. Restoration of transformation was independent of new RNA synthesis but sensitive to protein synthesis inhibitors, implying the existence of a heat-labile transforming protein. The other type of transformation-defective RSV mutants (td mutants) contained deletions of various sizes in the viral genomic RNA. In collaboration with Lu-Hai Wang and Duesberg, Kawai and Hanafusa used RNAase T1 mapping to determine the location of the proposed *Src* and *Env* genes on the retroviral genome (Wang et al., 1976). The stage was now set for momentous discoveries in molecular oncology. Dominique Stehelin, Harold Varmus, Michael Bishop, and Vogt prepared hybridization probes specific for the *Src* gene using td deletion mutants to deplete viral sequences unrelated to transformation. Using these probes, they made the exciting discovery that normal cells contained *Src*-related sequences.

Saburo and colleagues, now at Rockefeller University, used an elegant "marker-rescue" experiment inspired by bacteriophage genetics (Hanafusa et al., 1977) to search for a cellular *Src* gene. This experiment both recapitulated the likely evolution of RSV and demonstrated the transforming potential of the cellular *Src* gene. Saburo found that chickens injected with transformation-defective (deletion) mutants developed tumors, with long latency and distant from the site of injection (in contrast to the rapid and local tumorigenesis produced by transformation-competent RSV). Replication-competent transforming viruses could be recovered from these tumors yet, intriguingly, produced foci that were morphologically distinct from the original RSV (Hanafusa et al., 1977), suggesting the generation of a new transforming gene. Saburo concluded that these "recovered viruses" had, by recombination, captured the cellular *Src* gene. The conclusion was soon cemented by oligonucleotide fingerprinting, restriction mapping with heteroduplex analysis, peptide mapping, and, definitively, DNA sequencing of the cellular locus (Takeya and Hanafusa, 1983). For this landmark work, Saburo shared the 1982 Lasker Award for Biomedical Research.

The 1980s saw dizzying progress. The Hanafusa lab identified many new viral oncogenes (*Yes*, *Fps*, *Ros*) and their cognate cellular genes. The laboratory also made important advances regarding the mechanism of transformation. RSV engineered to carry c-*Src* instead of v-*Src* was found to be nontransforming; mutagenesis and domain-swap experiments defined important regions for what is now known to be c-Src autoinhibition. Other studies defined a critical role for v-Src myristoylation and membrane targeting for transformation, foreshadowing the critical importance of membrane recruitment in receptor-tyrosine kinase signaling.

The close of the 1980s brought another breakthrough. Tony Pawson's laboratory made the critical observation that, in addition to their conserved kinase (SH1) domains, Src and Fps shared other regions of sequence similarity, which they termed "Src Homology-2 (SH2)" and "Src Homology-3 (SH3)" domains. However, the function of these domains was obscure. Bruce Mayer, then a graduate student in the Hanafusa lab, cloned and sequenced the transforming gene of CT10 (v-*Crk*). Surprisingly, it encoded a fusion of a viral Gag protein with an SH2 domain and an SH3 domain (Mayer et al., 1988). Thus, the SH2 and SH3 domains alone could be the business end of an oncoprotein. Subsequent studies from the group showed that v-Crk expression increased cellular phosphotyrosine levels, and that v-Crk bound to multiple phosphotyrosyl proteins, at least one of which was a tyrosine kinase. Furthermore, the SH2, and to a lesser extent the SH3, domains were required for transformation and the SH2 domain was necessary for binding to phosphotyrosine-containing proteins. Contemporaneous and subsequent studies from the Pawson lab and from Mayer (first with Hanafusa and later as a postdoctoral fellow with David Baltimore) showed directly that SH2 domains bind to phosphotyrosine-containing proteins. These studies established the paradigm that modular protein-protein interaction domains are building blocks of cellular signaling pathways. In 1993, for these and other contributions to the understanding of oncogenic signaling, Saburo received the Alfred P. Sloan Prize from the General Motors Cancer Research Foundation.

The discovery of SH2 and SH3 domains also brought the long history of Rous sarcoma virus and the molecular basis of its transforming activity full circle. The recombination junction between c-Src and retroviral sequences replaced 19 amino acids from c-Src, which most likely occurred in the initial recombination event that generated the virus isolated by Rous in 1911. Loss of this C-terminal sequence was itself sufficient for turning c-Src into an oncogene. Later biochemical experiments and c-Src crystal structures explained this: the c-Src C-terminal tail contained a tyrosine that, when phosphorylated, bound intramolecularly to the SH2 domain, inactivating the c-Src kinase.

Saburo received many other honors, including the Order of Culture (the highest honor in Japan), the Howard Taylor Ricketts Award, the G.H. Clowes Memorial award from the American Association for Cancer Research, and a Doctorate of Science, honoris causa, from Rockefeller. He also was a Foreign Associate of the US National Academy of Sciences and a member of the Japanese Academy of Sciences. He received the Japan Cultural Merit Award and was an honorary member of the Japanese Societies for Virology and Cancer Research.

The scientific community will remember Hanafusa for his seminal work, but his students will surely remember him even more for his mentorship. He was a lover of ideas, with a profound appreciation of carefully executed and controlled experiments. Saburo was not a big talker; exciting and instructive conver-

sations happened, typically late at night and on weekends, but usually these conversations occurred while working side-by-side at the bench. Often they were initiated by his laconic and somewhat playful query: "What it means?"

Given how quiet and reserved Saburo was, the degree of loyalty and affection he inspired is remarkable. Again deeds, not words, carried the day. He cared about his trainees and we knew it; he looked after the little things that mattered to people. His house was often a jumbled way station for furniture being passed from one postdoc to the next. His laboratory was a melting pot for scientists from all over the world. We came together, experienced each other's cultures, did exciting science, and formed lifelong friendships.

Although Saburo's work ethic was legendary, he did have outside interests. He was dedicated to his daughter Kei, now a physician in San Francisco, as well as to his four grandchildren. He loved Beethoven, baseball, American football, Japanese sake, and a good whisky. He was an avid and highly competitive softball player; rumor had it that he was a star baseball pitcher during his youth. By sheer force of personality he would corral all lab members, independent of cultural background or athletic prowess, to play softball at least once every summer. The results were frequently comical, but he was too kind to laugh out loud.

After Teruko's untimely death in 1996, Saburo returned to Japan in 1998 as Director of the Osaka Bioscience Institute. In Osaka, he had the good fortune to meet his second wife, Emiko. Though slowed by illness, he continued to contribute to our understanding of oncogenesis while inspiring a new generation of Japanese scientists. He kept in touch with his former trainees and colleagues from the US, both remotely and at lab reunions held at the Osaka Bioscience Institute.

It is both ironic and cruel that Saburo, having contributed so much to our understanding of virology and oncogenesis, succumbed to liver cancer, a virus-associated malignancy. Yet there is much to celebrate. He dedicated his career to a single biological problem and, as a result of his intense focus and dedication, had the satisfaction of playing a major role in solving it. He lived to see new cancer therapies developed directly from the Oncogene Revolution that he helped to create. He was a scientist's scientist, publicity-shy, but, nevertheless, a widely recognized international figure. He was genuinely beloved by students, fellows, and colleagues. We all have to answer Saburo's question of "what it means?" in our own way. But Hidesaburo Hanafusa answered it in a way that many of us could only hope to emulate.

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